## ABSTRACT

The present invention provides a simple method for producing a dumbbell-shaped DNA.

A method for producing a dumbbell-shaped DNA, wherein each of sense and antisense strands is connected at both the 5' and 3' ends of a linear-shaped double stranded DNA by a single stranded DNA of loop structure, comprising the steps of;

1) amplifying a target DNA in a template DNA by PCR using sense and antisense primers, wherein each of the sense and antisense primers contains the following sequence (a) at the 5' end and also contains the following sequences (b), (c), and (d) in order from the 5' end to the 3' end,

(a) a part of a sense sequence of a nickase recognition sequence, comprising the sequence of a region between the site where a nick is introduced by the action of a nickase and the 3' end,

(b) a sequence capable of forming a loop structure from a single strand,

(c) the entire antisense sequence of the nickase recognition sequence (a),

(d) a sequence complementary to all or part of the sequence of the target DNA;

2) treating the amplified DNA product of step 1) with a nickase of (a);

3) heating and then annealing the nickase treated amplified DNA product of step 2); and

4) treating the heated and annealed amplified DNA product of step 3) with DNA ligase,

wherein the sense and antisense primers used in step 1) are phosphorylated at the 5' end, or the amplified DNA product is phosphorylated at the 5' end after step 1) but before step 4).